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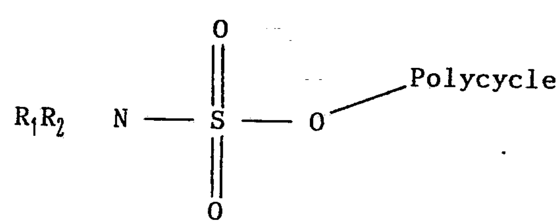
(54) Title: STEROID SULPHATASE INHIBITORS



(I)

(57) Abstract

Novel steroid sulphatase inhibitors are disclosed as well as pharmaceutical compositions containing them for use in the treatment of oestrone dependent tumours, especially breast cancer. The novel steroid sulphatase inhibitors are: sulphamate esters of formula (I), where R_1 and R_2 are each H, alkyl, alkenyl, cycloalkyl or aryl, or together represent an alkylene group optionally containing a heteroatom e.g. -O- or -NH-; and -O-polycycle represents the residue of a polycyclic alcohol, preferably a sterol, most preferably a 3-sterol. Preferred compounds are oestrone-3-sulphamate and N,N-dimethyl oestrone-3-sulphamate.

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STEROID SULPHATASE INHIBITORS

FIELD OF INVENTION

This invention relates to novel compounds for use as steroid sulphatase inhibitors, and pharmaceutical compositions containing them.

BACKGROUND AND PRIOR ART

Steroid precursors, or pro-hormones, having a sulphate group in the 3-position of the steroid nucleus, referred to hereinafter simply as steroid sulphates, are known to play an important part as intermediates in steroid metabolism in the human body. Oestrone sulphate and dehydroepiandrosterone (DHA) sulphate, for example, are known to play an important role as intermediates in the production, in the body, of oestrogens such as oestrone and oestradiol. Oestrone sulphate, in particular, is known, for example, to represent one of the major circulating oestrogen precursors particularly in post-menopausal women and oestrone sulphatase activity in breast tumours is 100-1000 fold greater than that of other enzymes involved in oestrogen formation (James et al., *Steroids*, 50, 269-279 (1987)).

Not only that, but oestrogens such as oestrone and oestradiol, particularly the over-production thereof, are strongly implicated in malignant conditions, such as breast cancer, see *Breast Cancer, Treatment and Prognosis*: Ed. R.A. Stoll, pp. 156-172, Blackwell Scientific Publications (1986), and the control of oestrogen production is the specific target of many anti-cancer therapies, both chemotherapy and surgical, e.g. oöphorectomy and adrenalectomy. So far as endocrine therapy is concerned, efforts have so far tended to concentrate on aromatase inhibitors, i.e. compounds which inhibit aromatase activity, which activity is involved, as the accompanying oestrogen metabolic flow diagram (Figure 1) shows, in the conversion of androgens such as androstenedione and testosterone to oestrone and oestradiol respectively.

In recently published International Application WO91/13083 a proposal has been made to target a different point in the oestrogen metabolic pathway, or rather two different points, that is to say the conversion of DHA sulphate and oestrone sulphate to DHA and oestrone, respectively, by steroid sulphatase activity, and using 3-monoalkyl-

thiophosphonate steroid esters as a steroid sulphatase inhibitor, more especially oestrone-3-monomethylthiophosphonate.

OBJECTS OF THE INVENTION

A first object of the present invention is to provide new compounds capable of inhibiting steroid sulphatase activity *in vitro* and *in vivo*.

A second object of the present invention is to provide new compounds having improved activity as steroid sulphatase inhibitors both *in vitro* and *in vivo*.

A third object of the invention is to provide pharmaceutical compositions effective in the treatment of oestrogen dependent tumours.

A fourth object of the invention is to provide pharmaceutical compositions effective in the treatment of breast cancer.

A fifth object of the invention is to provide a method for the treatment of oestrogen dependent tumours in mammals, especially humans.

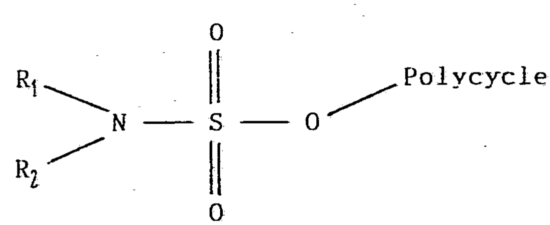
A sixth object of the invention is to provide a method for the treatment of breast cancer in mammals and especially in women.

SUMMARY OF INVENTION

The invention is based on the discovery of novel compounds having steroid sulphatase inhibitory activity, in some cases, with extremely high activity levels. These compounds are the sulphamic acid esters of polycyclic alcohols, being polycyclic alcohols the sulphate of which is a substrate for enzymes having steroid sulphatase (EC 3.1.6.2) activity, the N-alkyl and N-aryl derivatives of those sulphamic acid esters, and their pharmaceutically acceptable salts.

Broadly speaking, the novel compounds of this invention are compounds of the Formula (I)

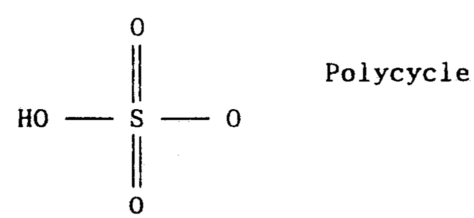
FORMULA (I)



where: R_1 and R_2 are each independently selected from H, alkyl, cycloalkyl, elkenyl and aryl, or together represent alkylene optionally containing one or more hetero atoms or groups in the alkylene chain; and

the group -O- polycycle represents the residue of a polycyclic alcohol, the sulphate of which is a substrate for enzymes having steroid sulphatase activity (EC 3.1.6.2).

As used herein the reference to polycyclic alcohols, the sulphate of which is a substrate for enzymes having steroid sulphatase activity refers to polycyclic alcohols, the sulphate of which, viz: the derivatives of the Formula:



which when incubated with steroid sulphatase EC 3.1.6.2 at pH 7.4 and 37°C and provides a K_m value of less than 50μmoles.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a schematic chart showing the metabolic pathways, enzymes and steroid intermediates associated with the production of oestradiol *in vivo*.

The activity of the present compounds as steroid sulphatase inhibitors is illustrated in the accompanying drawings:

Figure 2 is a histogram showing the dose-dependent inhibitory effect of oestrone-3-sulphamate on steroid sulphatase activity in human MCF-7 cells *in vitro*.

Figure 3 is a histogram showing the dose-dependent inhibitory effect of oestrone-3-N,N-dimethylsulphamate on steroid sulphatase activity in human MCF-7 cells *in vitro*.

Figure 4 is a graph comparing the log dose-response curves for oestrone-3-sulphamate and oestrone-3-N,N-dimethylsulphamate on steroid sulphatase activity in human MCF-7 cells *in vitro*.

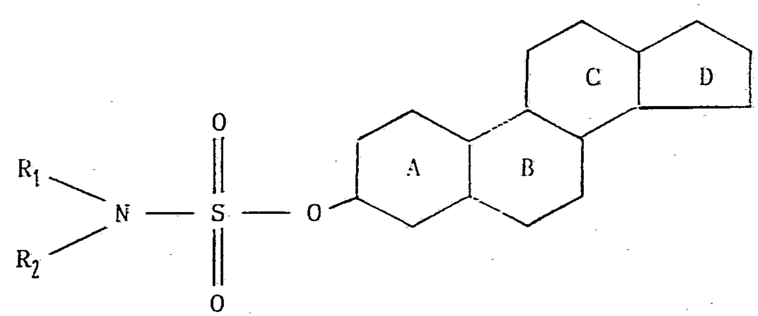
Figure 5 is a graph showing the dose-dependent inhibitory effect of oestrone-3-sulphamate, together with its IC_{50} value (concentration required to produce 50% inhibition), on steroid sulphatase activity in human placental microsomes *in vitro*.

DETAILED DESCRIPTION

In one aspect the present invention provides, as novel compounds, the sulphamic acid esters of polycyclic alcohols, being polycyclic alcohols the sulphate of which is a substrate for enzymes having steroid sulphatase activity in accordance with the definition already provided, and their N-alkyl, N-cycloalkyl, N-alkenyl and N-aryl derivatives. These compounds are of Formula I hereinbefore given.

Preferably the polycyclic group will contain, inclusive of all substituents, a maximum of about 40 carbon atoms, more usually no more than about 30. Preferred polycycles are those containing a steroidal ring structure, that is to say a cyclopentanophenanthrene skeleton. Preferably, the sulphamyl or substituted sulphamyl group is attached to that skeleton in the 3-position, that is to say are compounds of the Formula II:

FORMULA (II)



where R_1 and R_2 are as above defined and the ring system ABCD represents a substituted or unsubstituted, saturated or unsaturated steroid nucleus, preferably oestrone or dehydroepiandrosterone.

Other suitable steroid ring systems are:

substituted oestrones, viz:

2-OH-oestrone 2-methoxy-oestrone 4-OH-oestrone 6α-OH-oestrone 7α-OH-oestrone 16α-OH-oestrone 16β-OH-oestrone

oestradiols and substituted oestradiols, viz:

2-OH-17ß-oestradiol 2-methoxy-17ß-oestradiol 4-OH-17ß-oestradiol
6α-OH-17ß-oestradiol 7α-OH-17ß-oestradiol 16α-OH-17α-oestradiol
16ß-OH-17α-oestradiol 16ß-OH-17ß-oestradiol 17α-oestradiol
17ß-oestradiol 17α-ethinyl-17ß-oestradiol

oestriols and substituted oestriols, viz:

oestriol 2-OH-oestriol 2-methoxy-oestriol
4-OH-oestriol 6α-OH-oestriol 7α-OH-oestriol

substituted dehydroepiandrosterones, viz:

6α-OH-dehydroepiandrosterone 7α-OH-dehydroepiandrosterone
16α-OH-dehydroepiandrosterone 16ß-OH-dehydroepiandrosterone

In general terms the steroid ring system ABCD may contain a variety of non-interfering substituents. In particular, the ring system ABCD may contain one or more hydroxy, alkyl especially lower (C_1-C_6) alkyl, e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl and other pentyl isomers, and n-hexyl and other hexyl isomers, alkoxy especially lower (C_1-C_6) alkoxy, e.g. methoxy, ethoxy, propoxy etc., alkinyl, e.g. ethinyl, or halogen, e.g. fluoro substituents.

Other suitable non-steroidal ring systems include: diethylstilboestrol, stilboestrol and other ring systems providing sulfates having K_m values of less than 50μmoles with steroid sulphatase EC3.1.6.2.

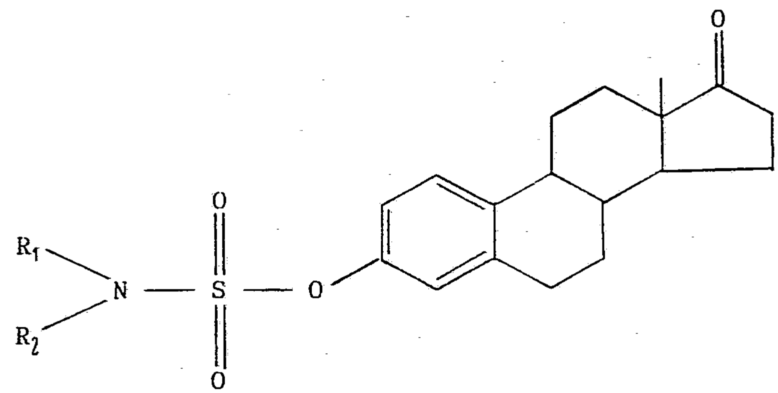
When substituted, the N-substituted compounds of this invention may contain one or two N-alkyl, N-alkenyl, N-cycloalkyl or N-aryl substituents, preferably containing or each containing a maximum of 10 carbon atoms. When R_1 and/or R_2 is alkyl, the preferred values are those where R_1 and R_2 are each independently selected from lower alkyl groups containing from 1 to 5 carbon atoms, that is to say methyl, ethyl, propyl etc. Preferably R_1 and R_2 are both methyl. When R_1 and/or R_2 is aryl, typical values are phenyl and tolyl ($-PhCH_3$; *o*-, *m*- or *p*-). Where R_1 and R_2 represent cycloalkyl, typical values are cyclopropyl, cyclopentyl, cyclohexyl etc. When joined together R_1 and R_2 typically represent an alkylene group providing a chain of 4 to 6 carbon atoms, optionally interrupted by one or more hetero atoms or groups, e.g. -O- or -NH- to provide a 5-, 6- or) - membered heterocycle, e.g. morpholino

pyrrolidono or piperidino.

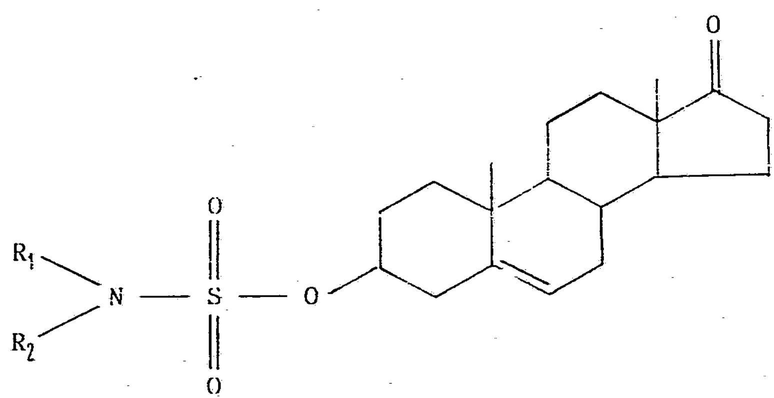
Within the values alkyl, cycloalkyl, alkenyl and aryl we include substituted groups containing as substituents therein one or more groups which do not interfere with the sulphatase inhibitory activity of the compound in question. Exemplary non-interfering substituents include hydroxy, amino, halo, alkoxy, alkyl and aryl.

Most preferred are compounds of the Formula III and IV:

FORMULA (III)



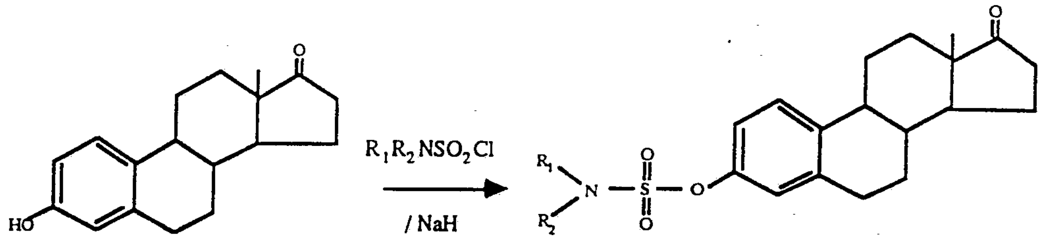
FORMULA (IV)



where R_1 and R_2 are H or C_1-C_5 alkyl, i.e. oestrone-3-sulphamate and dehydroepiandrosterone-3-sulphamate and their N-(C_1-C_5) alkyl derivatives, especially the dimethyl derivatives, $R_1 = R_2 = CH_3$.

The sulphamic acid esters of this invention are prepared by reacting the polycyclic alcohol, e.g. oestrone or dehydroepiandrosterone, with a sulfamoyl chloride $R_1R_2NSO_2Cl$, i.e. the reaction scheme I

REACTION SCHEME I



Oestrone

Conditions for carrying out reaction scheme I are as follows:

Sodium hydride and a sulphamoyl chloride are added to a stirred solution of oestrone in anhydrous dimethyl formamide at 0°C. Subsequently, the reaction is allowed to warm to room temperature whereupon stirring is continued for a further 24 hours. The reaction mixture is poured onto a cold saturated solution of sodium bicarbonate and the resulting aqueous phase is extracted with dichloromethane. The combined organic extracts are dried over anhydrous $MgSO_4$. Filtration followed by solvent evaporation *in vacuo* and co-evaporation with toluene affords a crude residue which is further purified by flash chromatography.

Where necessary, functional groups in the polycyclic alcohol (sterol) may be protected in known manner and the protecting group or groups removed at the end of the reaction.

For pharmaceutical administration, the steroid sulphatase inhibitors of this invention can be formulated in any suitable manner utilising conventional pharmaceutical formulating techniques and pharmaceutical carriers, exipients, diluents etc. and usually for parenteral administration. Approximate effective dose rates are in the range 100 to 800 mg/day depending on the individual activities of the compounds in question and for a patient of average (70kg) bodyweight. More usual dosage rates for the preferred and more active compounds will be in the range 200 to 800 mg/day, more preferably, 200 to 500 mg/day, most preferably from 200 to 250 mg/day. They may be given in single dose regimes, split dose regimes and/or in multiple dose regimes lasting over several days. For oral administration they may be formulated in tablets, capsules, solution or suspension containing from 100 to 500 mg of compound per unit dose. Alternatively and preferably

the compounds will be formulated for parenteral administration in a suitable parenterally administrable carrier and providing single daily dosage rates in the range 200 to 800 mg, preferably 200 to 500, more preferably 200 to 250 mg. Such effective daily doses will, however, vary depending on inherent activity of the active ingredient and on the bodyweight of the patient, such variations being within the skill and judgement of the physician.

For particular applications, it is envisaged that the steroid sulphatase inhibitors of this invention may be used in combination therapies, either with another sulphatase inhibitor, or, for example, in combination with an aromatase inhibitor, such as for example, 4-hydroxyandrostenedione (4-OHA).

The invention is illustrated by the following preparative Examples and test data:

Example 1

Preparation of oestrone-3-sulphamate

Sodium hydride (60% dispersion; 2 eq) and sulphamoyl chloride (2 eq) were added to a stirred solution of oestrone (1 eq) in anhydrous dimethyl formamide at 0°C. Subsequently, the reaction was allowed to warm to room temperature whereupon stirring was continued for a further 24 hours.

The reaction mixture was poured onto a cold saturated solution of sodium bicarbonate and the resulting aqueous phase was extracted with dichloromethane. The combined organic extracts were dried over anhydrous $MgSO_4$. Filtration followed solvent evaporation in vacuo and co-evaporation with toluene afforded a crude residue which is further purified by flash chromatography.

Analysis showed the following data:

δ^1H (270MHz; CD_3OD): 0.91 (s, 3H, C_{18}-Me), 1.40-2.55 (series of m, 13H), 2.90-2.92 (m, 2H), 7.04 (br d, 2H, J=10.44Hz), 7.33 (br d, 1H, J=8.42Hz).

$\delta^{13}C$ (67.8MHz; CD_3OD): 14.53 (q, C_{18}-Me), 22.80 (t), 27.24 (t), 27.73 (t), 30.68 (t), 33.05 (t), 37.01 (t), 39.76 (d), 45.73 (s, C_{18}), 51.86

(d), 120.76 (d), 123.54 (d), 127.89 (d), 139.83 (s), 150.27 (s), 223.87 (s, C=O).

m/z (%): 349 (9) (m^+), 270 (100), 213 (26), 185 (43), 172 (31), 159 (21), 146 (36), 91 (33), 69 (37), 57 (73), 43 (56), 29 (24).

Microanalysis:

	C	H	N
Expected:	61.87%	6.63%	4.01%
Found:	61.90%	6.58%	3.95%

Example 2

Preparation of oestrone-3-N-methylsulphamate

The procedure of Example 1 was repeated save that sulphamoyl chloride was replaced by the same quantity of N-methylsulphamoyl chloride.

Analysis showed the following data:

δ^1H (270MHz; $CDCl_3$): 0.91 (s, 3H, C_{18}-Me), 1.28-1.68 (m, 6H), 1.93-2.60 (series of m, 7H), 2.90-2.95 (m, 2H), 2.94 (d, 3H, J=5.13 Hz, MeN-), 4.68-4.71 (br m, exchangeable, 1H, -NH), 7.02-7.07 (m, 2H), 7.26-7.32 (m, 1H).

m/z (%): 364 $[M+H]^+$

Example 3

Preparation of oestrone-3-N,N-dimethylsulphamate

The procedure of Example 1 was repeated save that sulphamoyl chloride was replaced by the same quantity of N,N-dimethylsulphamoyl chloride.

Analysis showed the following data:

δ^1H (270MHz; $CDCl_3$): 0.92 (s, 3H, C_{18}-Me), 1.39-1.75 (m, 5H), 1.95-2.60 (series of m, 6H), 2.82 (s, 3H, MeN-), 2.96-3.00 (m, 4H), 2.98 (s, 3H, MeN-), 7.04 (br d, 2H, J=7.69Hz), 7.29 (br d, 1H, J=7.88Hz).

m/z (%): 377 $[M]^+$

Microanalysis:

	C	H	N
Expected:	63.63%	7.21%	3.71%
Found:	63.50%	7.23%	3.60%

Example 4

Inhibition of Steroid Sulphatase Activity in MCF-7 cells by oestrone-3-sulphamate

Steroid sulphatase is defined as: Steryl Sulphatase EC 3.1.6.2.

Steroid sulphatase activity was measured *in vitro* using intact MCF-7 human breast cancer cells. This hormone dependent cell line is widely used to study the control of human breast cancer cell growth. It possesses significant steroid sulphatase activity (MacIndoe et al. *Endocrinology*, 123, 1281-1287 (1988); Purohit & Reed, *Int. J. Cancer*, 50, 901-905 (1992)) and is available in the U.S.A. from the American Type Culture Collection (ATCC) and in the U.K. (e.g. from The Imperial Cancer Research Fund). Cells were maintained in Minimal Essential Medium (MEM) (Flow Laboratories, Irvine, Scotland) containing 20 mM HEPES, 5% foetal bovine serum, 2 mM glutamine, non-essential amino acids and 0.075% sodium bicarbonate. Up to 30 replicate 25 cm^2 tissue culture flasks were seeded with approximately 1 x 10^5 cells/flask using the above medium. Cells were grown to 80% confluency and medium was changed every third day.

Intact monolayers of MCF-7 cells in triplicate 25 cm^2 tissue culture flasks were washed with Earle's Balanced Salt Solution (EBSS from ICN Flow, High Wycombe, U.K.) and incubated for 3-4 hours at 37°C with 5 pmol (7 x 10^5 dpm) $[6,7-^3H]$oestrone-3-sulphate (specific activity 60 Ci/mmol from New England Nuclear, Boston, Mass., U.S.A.) in serum-free MEM (2.5 ml) together with oestrone-3-sulphamate (11 concentrations: 0; 1fM; 0.01pM; 0.1pM; 1pM; 0.01nM; 0.1nM; 1nM; 0.01μM; 0.1μM; 1μM). After incubation each flask was cooled and the medium (1 ml) was pipetted into separate tubes containing $[^{14}C]$oestrone (7 x 10^3 dpm) (specific activity 97 Ci/mmol from Amersham International Radiochemical Centre, Amersham, U.K.). The mixture was shaken thoroughly for 30 seconds with toluene (5 ml). Experiments showed that

>90% [^{14}C]oestrone and <0.1% [^{3}H]oestrone-3-sulphate was removed from the aqueous phase by this treatment. A portion (2 ml) of the organic phase was removed, evaporated and the ^{3}H and ^{14}C content of the residue determined by scintillation spectrometry. The mass of oestrone-3-sulphate hydrolysed was calculated from the ^{3}H counts obtained (corrected for the volumes of the medium and organic phase used, and for recovery of [^{14}C]oestrone added) and the specific activity of the substrate. Each batch of experiments included incubations of microsomes prepared from a sulphatase-positive human placenta (positive control) and flasks without cells (to assess apparent non-enzymatic hydrolysis of the substrate). The number of cell nuclei per flask was determined using a Coulter Counter after treating the cell monolayers with Zaponin. One flask in each batch was used to assess cell membrane status and viability using the Trypan Blue exclusion method (Phillips, H.J. (1973) In: *Tissue culture and applications*, [eds: Kruse, D.F. & Patterson, M.K.]; pp. 406-408; Academic Press, New York).

Data for oestrone-3-sulphamate are shown in Table I and Figures 2 and 4. Results for steroid sulphatase activity are expressed as the mean ± 1 S.D. of the total product (oestrone + oestradiol) formed during the incubation period (20 hours) calculated for 10^6 cells and, for values showing statistical significance, as a percentage reduction (inhibition) over incubations containing no oestrone-3-sulphamate. Unpaired Student's t-test was used to test the statistical significance of results.

TABLE I

Steroid Sulphatase Activity in MCF-7 cells in the presence of Oestrone-3-sulphamate		
Oestrone-3-sumphamate concentration	Steroid Sulphatase Activity ¶ (fmol/20 hr/10^6 cells)	% reduction over control (% inhibition)
0 (control)	319.7 ± 18.5	-
1fM	353.3 ± 39.0	-
0.01pM	362.3 ± 21.2	-
0.1pM	330.7 ± 17.8	-
1pM	321.8 ± 6.2	-
0.01nM	265.1 ± 11.0*	17.2%
0.1nM	124.8 ± 12.4***	60.9%
1nM	16.49 ± 4.7***	95.0%
0.01μM	3.92 ± 0.4***	98.8%
0.1μM	2.53 ± 1.1***	99.2%
1μM	1.68 ± 0.7***	99.5%

¶ mean ± 1 S.D. n=3 * p ≤0.05 *** p≤0.001

Example 5

Inhibition of Steroid Sulphatase Activity in MCF-7 cells by oestrone-3-N,N-dimethylsulphamate

An identical experimental protocol to that described in Example 4 was used to generate results for oestrone-3-N,N-dimethylsulphamate except that incubations contained oestrone-3-N,N-dimethylsulphamate (5 concentrations: 0; 0.001μM; 0.01μM; 0.1μM; 1μM) in place of oestrone-3-sulphamate.

Results for oestrone-3-N,N-dimethylsulphamate are shown in Table II and Figure 3 and are expressed in an identical manner to Table I and Figure 2 respectively. Additionally the log dose-response curve is compared with oestrone-3-sulphamate in Figure 4.

TABLE II

Steroid Sulphatase Activity in MCF-7 cells in the presence of oestrone-3-N,N-dimethylsulphamate		
Oestrone-3-N,N-dimethylsulphamate concentration	Steroid Sulphatase Activity ¶ (fmol/20 hr/10^6 cells)	% reduction over control (% inhibition)
0 (control)	82.63 ± 3.6	-
0.001μM	68.33 ± 3.2**	17.3%
0.01μM	46.0 ± 4.9***	44.3%
0.1μM	17.43 ± 4.3***	78.9%
1μM	11.89 ± 3.7***	85.6%

¶ mean ± 1 S.D. n=3   ** $p \leq 0.01$ *** $p \leq 0.001$

Example 6

Inhibition of Steroid Sulphatase Activity in MCF-7 cells by pre-treatment with oestrone-3-N,N-dimethylsulphamate and oestrone-3-N,N-dimethylsulphamate

A similar experimental protocol to that described in Example 4 was used to determine the effect of pre-treating MCF-7 cells with oestrone-3-sulphamate and oestrone-3-N,N-dimethylsulphamate respectively.

Intact monolayers were initially incubated for 2 hours at 37°C with 0.1 μM oestrone-3-sulphamate, oestrone-3-N,N-dimethylsulphamate or medium alone (control). The medium bathing the cells was then removed by aspiration and cells were washed 3 times successively with 5 ml of medium on each occasion. The resultant 'washed' cells were then re-suspended and incubated for 3-4 hours at 37°C in medium containing 5 pmol (7 x 10^5 dpm) [6,7-^{3}H]oestrone-3-sulphate. All other aspects were identical to those described in Examples 3 and 4.

Results for oestrone-3-sulphamate and oestrone-3-N,N-dimethyl-sulphamate are shown in Table III and are expressed in a similar manner to Table I.

TABLE III

Steroid Sulphatase Activity in MCF-7 cells pre-incubated with oestrone-3-sulphamates

Pre-treatment	Steroid Sulphatase Activity ¶ (fmol/20 hr/10^6 cells)	% reduction over control (% inhibition)
Control	65.4 ± 6.4	-
Oestrone-3-sulphamate	1.7 ± 0.2***	97.4%
Oestrone-3-N,N-dimethylsulphamate	53.1 ± 3.4*	18.8%

¶ mean ± 1 S.D. n=3   * p ≤0.05   *** p ≤0.001

Example 7

Inhibition of Steroid Sulphatase Activity in Placental Microsomes by Oestrone-3-sulphamate

Sulphatase-positive human placenta from normal term pregnancies (Obstetric Ward, St. Mary's Hospital, London) were thoroughly minced with scissors and washed once with cold phosphate buffer (pH 7.4, 50 mM) then re-suspended in cold phosphate buffer (5 ml/g tissue). Homogenisation was accomplished with an Ultra-Turrax homogeniser, using three 10 second bursts separated by 2 minute cooling periods in ice. Nuclei and cell debris were removed by centrifuging (4°C) at 2000g for 30 minutes and portions (2 ml) of the supernatant were stored at -20°C. The protein concentration of the supernatants was determined by the method of Bradford (*Anal. Biochem.*, 72, 248-254 (1976)).

Incubations (1 ml) were carried out using a protein concentration of 100 µg/ml, substrate concentration of 20 µM [6,7-^{3}H]oestrone-3-sulphate (specific activity 60 Ci/mmol from New England Nuclear, Boston, Mass., U.S.A.) and an incubation time of 20 minutes at 37°C. Eight concentrations of oestrone-3-sulphamate were employed: 0 (i.e. control); 0.05µM; 0.1µM; 0.2µM; 0.4µM; 0.6µM; 0.8µM; 1.0µM. After incubation each sample was cooled and the medium (1 ml) was pipetted into separate tubes containing [^{14}C]oestrone (7 x 10^3 dpm) (specific activity 97 Ci/mmol from Amersham International Radiochemical Centre, Amersham, U.K.). The mixture was shaken thoroughly for 30

seconds with toluene (5 ml). Experiments showed that >90% [^{14}C]oestrone and <0.1% [^{3}H]oestrone-3-sulphate was removed from the aqueous phase by this treatment. A portion (2 ml) of the organic phase was removed, evaporated and the ^{3}H and ^{14}C content of the residue determined by scintillation spectrometry. The mass of oestrone-3-sulphate hydrolysed was calculated from the ^{3}H counts obtained (corrected for the volumes of the medium and organic phase used, and for recovery of [^{14}C]oestrone added) and the specific activity of the substrate.

Results for oestrone-3-sulphamate are shown in Table IV and Figure 5. Results for steroid sulphatase activity are expressed in Table IV as total product (oestrone + oestradiol) formed during the incubation period (time) and as a percentage reduction (inhibition) over incubations containing no oestrone-3-sulphamate which acted as control. Results for steroid sulphatase activity are expressed in Figure 4 as percentage reduction (inhibition) over control against concentration of oestrone-3-sulphamate and include the calculated IC_{50} value (i.e. the concentration of oestrone-3-sulphamate which produces 50% inhibition in relation to control) of 0.07μM.

TABLE IV

Steroid Sulphatase Activity in placental microsomes in the presence of Oestrone-3-sulphamate

Oestrone-3-sulphamate concentration	Steroid Sulphatase Activity ¶ (pmol/hr/0.1 mg protein)	% reduction over control (% inhibition)
0 (control)	768.6	-
0.05μM	430.4	44.0%
0.1μM	305.9	60.2%
0.2μM	140.0	81.8%
0.4μM	83.3	89.2%
0.6μM	61.8	92.0%
0.8μM	49.2	93.6%
1.0μM	51.6	93.3%

¶ mean of 2 estimates

Example 8

Inhibition of Steroid Sulphatase Activity in Liver Microsome Preparations from Rats treated with subcutaneous Oestrone-3-sulphamate

Four groups of 3 female Wistar rats (weight range 80-110g) were given 100 µl subcutaneous injections (once daily for 7 days, vehicle: propylene glycol) of either:

Propylene glycol (vehicle control)

Oestrone-3-sulphamate (10 mg/kg/day)

Oestrone-3-sulphate (10 mg/kg/day) (substrate control)

Oestrone-3-sulphate (10 mg/kg/day) + Oestrone-3-sulphamate (10 mg/kg/day)

On the eighth day all rats were sacrificed and livers were removed by dissection. Liver microsomal preparations were prepared by an identical protocol to that described in Example 6 except that the tissue source was rat liver and that duplicate experiments to determine steroid sulphatase activity were performed using [6,7-^{3}H]oestrone-3-sulphate and [7-^{3}H]dehydroepiandrosterone-3-sulphate as separate substrates.

Results for steroid sulphatase activity are shown in Table V and are expressed as total product formed during the incubation period in the form of mean ± 1 S.D. Results for incubations of tissue obtained from groups of rats treated with oestrone-3-sulphamate are also expressed as a percentage reduction (inhibition) in steroid sulphatase activity compared to their respective controls.

TABLE V

Steroid Sulphatase Activity in Liver Microsome Preparations from Rats treated with subcutaneous Oestrone-3-sulphamate

Treatment Group	Assay Substrate	Steroid Sulphatase Activity ¶ (nmol/30 min/200 µg protein)	% reduction over control
control (vehicle)	E_1-S	20.95 ± 0.2	-
E_1-SO_3NH_2	E_1-S	0.34 ± 0.1***	98.4%
control (E_1-S)	E_1-S	20.6 ± 0.4	-
E_1-S + E_1-SO_3NH_2	E_1-S	0.21 ± 0.03***	99.0%
control (vehicle)	DHA-S	1.73 ± 0.4	-
E_1-SO_2NH_2	DHA-S	0.1 ± 0.01***	94.2%
control (E_1-S)	DHA-S	1.71 ± 0.1	-
E_1-S + E_1-SO_3NH_2	DHA-S	0.09 ± 0.01***	94.7%

¶ mean ± 1 S.D. n=3

*** $p \leq 0.001$

E_1-S = oestrone-3-sulphamate

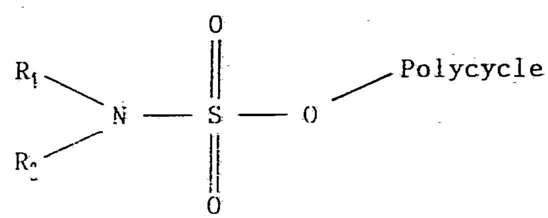
DHA-S = dehydroepiandrosterone-3-sulphate

E_1-SO_2NH_2 = oestrone-3-N,N-dimethylsulphamate

CLAIMS

1. The sulphamic acid esters of polycyclic alcohols, being polycyclic alcohols the sulphate of which is a substrate for enzymes having steroid sulphatase activity, and their N-alkyl, N-alkenyl, N-cycloalkyl and N-aryl derivatives.

2. Sulphamic acid esters according to claim 1 which are of the Formula



where R_1 and R_2 are each independently selected from H, alkyl, alkenyl, cycloalkyl and aryl, or together represent alkylene optionally containing one or more hetero atoms or groups in the alkylene chain; and

the group -O-polycycle represents the said residue of the polycyclic alcohol, the sulphate ester of which is a substrate for enzymes having steroid sulphatic activity (EC 3.1.6.2).

3. Sulphamic acid esters according to claim 2, wherein the said polycyclic alcohol is a sterol.

4. Sulphamic acid esters according to claim 3, wherein the said sterol is a 3-sterol.

5. Sulphamic acid esters according to claim 4, wherein the said sterol is selected from the group consisting of oestrone, dehydroepiandrosterones, substituted oestrones and substituted dehydroepiandrosterones.

6. Sulphamic acid esters according to any one of claims 2 to 5, being the N-alkyl substituted compounds wherein the N-alkyl

substituent(s) is or are C_1-C_{10} alkyl.

7. Sulphamic acid esters according to claim 6, wherein the N-alkyl substituent(s) is or are C_1-C_5 alkyl.

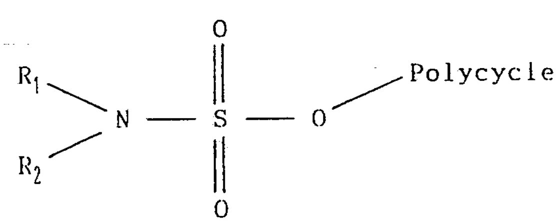
8. Sulphamic acid esters according to claim 6, wherein the N-alkyl substituent(s) is or are methyl group(s).

9. Oestrone 3-sulphamate.

10. Oestrone-3-N,N-dimethylsulphamate.

11. A pharmaceutical preparation for the treatment of oestrogen dependent tumours comprising a steroid sulphatase inhibitor in admixture with a pharmaceutically acceptable diluent or carrier, wherein the steroid sulphatase inhibitor is or comprises an effective amount of a sulphamic acid ester as claimed in any one of claims 1 to 10.

12. In a method for the treatment of oestrogen dependent tumours in mammals, which comprises administering to the mammal, optionally in admixture with or in conjunction with one or more other chemotherapeutic or other pharmaceutically active compounds as part of a combination therapy regime, an inhibitor of steroid sulphatase activity *in vivo*, the improvement which comprises using as the steroid sulphatase inhibitor an effective amount of a compound of the formula



where R_1 and R_2 are each independently selected from H, alkyl, alkenyl, cycloalkyl and aryl, or together represent alkylene optionally containing one or more heteroatoms or groups in the alkylene chain; and

the group -O-polycycle represents the said residue of the polycyclic alcohol, the sulphate ester of which is a substrate for enzymes having steroid sulphatase activity (EC 3.1.6.2).

13. A method according to claim 12, wherein in the formula of said steroid sulphatase inhibitor, the group O-polycycle represents a 3-sterol residue.

14. A method according to claim 13, wherein said 3-sterol residue is selected from 3-oestrone and 3-dehydroepiandrosterone residues.

15. A method according to claim 12, wherein the steroid sulphatase inhibitor is selected from the group consisting of oestrone-3-sulphamate, oestrone-3-(C_1-C_6)alkyl sulphamate, dehydroepiandrosterone-3-sulphamate, and dehydroepiandrosterone-3-(C_1-C_6)alkyl sulphamate.

16. A method according to claim 12, wherein the steroid sulphatase inhibitor is selected from the group consisting of oestrone-3-sulphamate, oestrone-3-N,N-dimethyl sulphamate, and oestrone-3-N-monomethyl sulphamate.

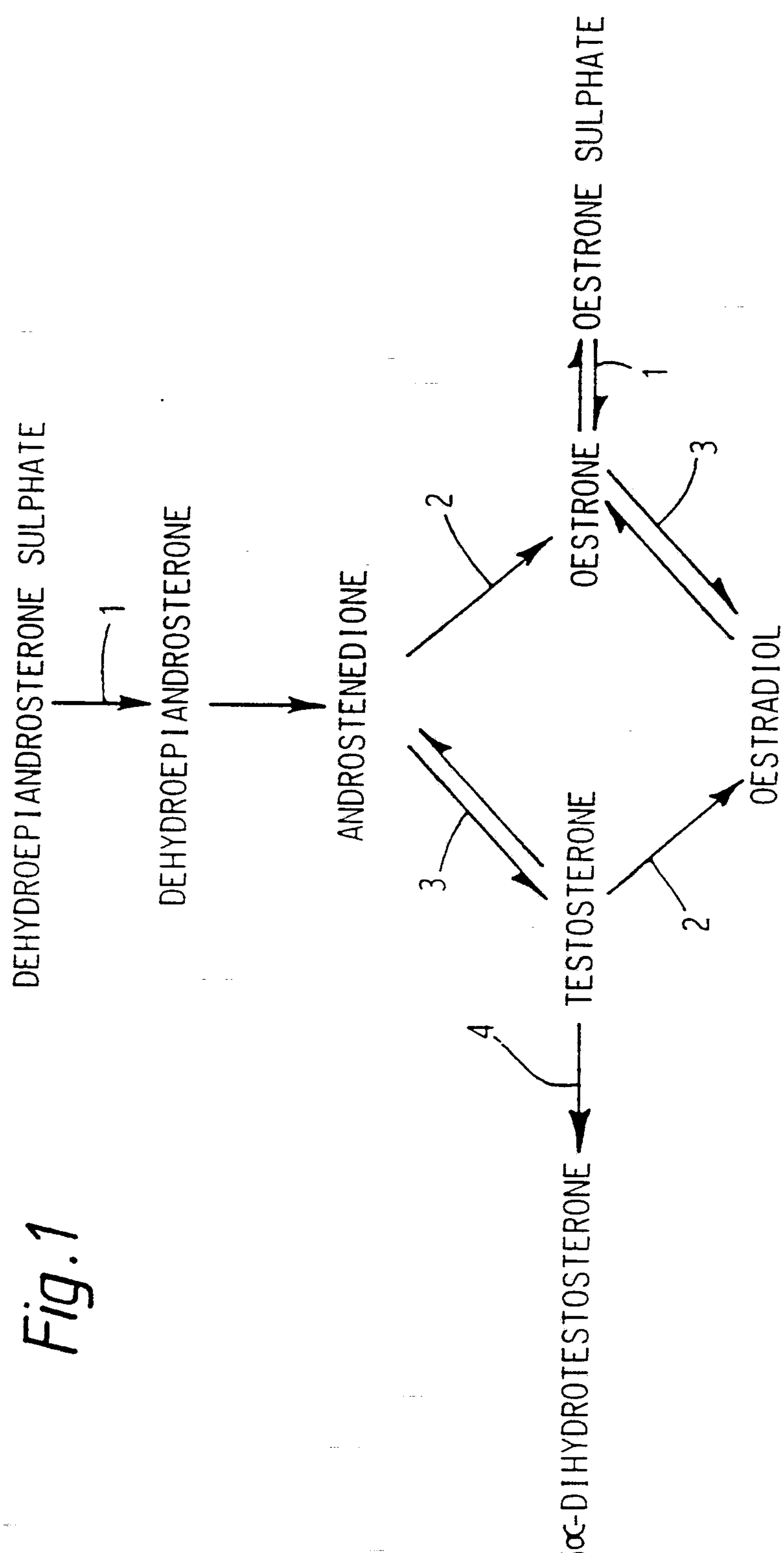


Fig.1

KEY ENZYMES IN STEROIDOGENESIS:-
1. SULPHATASE 2. AROMATASE 3. DEHYDROGENASE 4. 5α REDUCTASE

Fig. 2

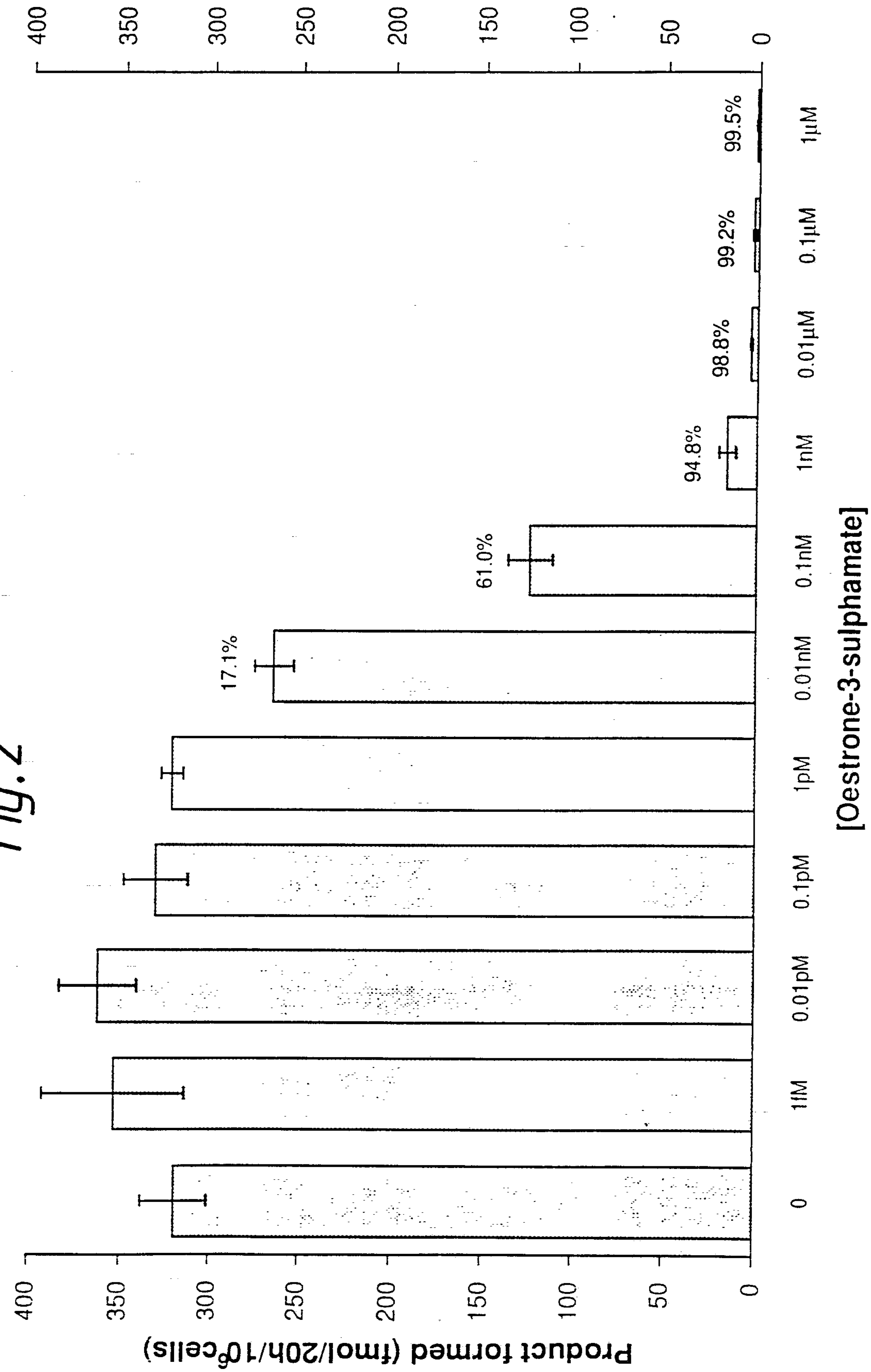

Product formed (fmol/20h/10⁶cells)
0
50
100
150
200
250
300
350
400
0
1fM
0.01pM
0.1pM
1pM
0.01nM
0.1nM
1nM
0.01μM
0.1μM
1μM
17.1%
61.0%
94.8%
98.8%
99.2%
99.5%
[Oestrone-3-sulphamate]

Fig. 3

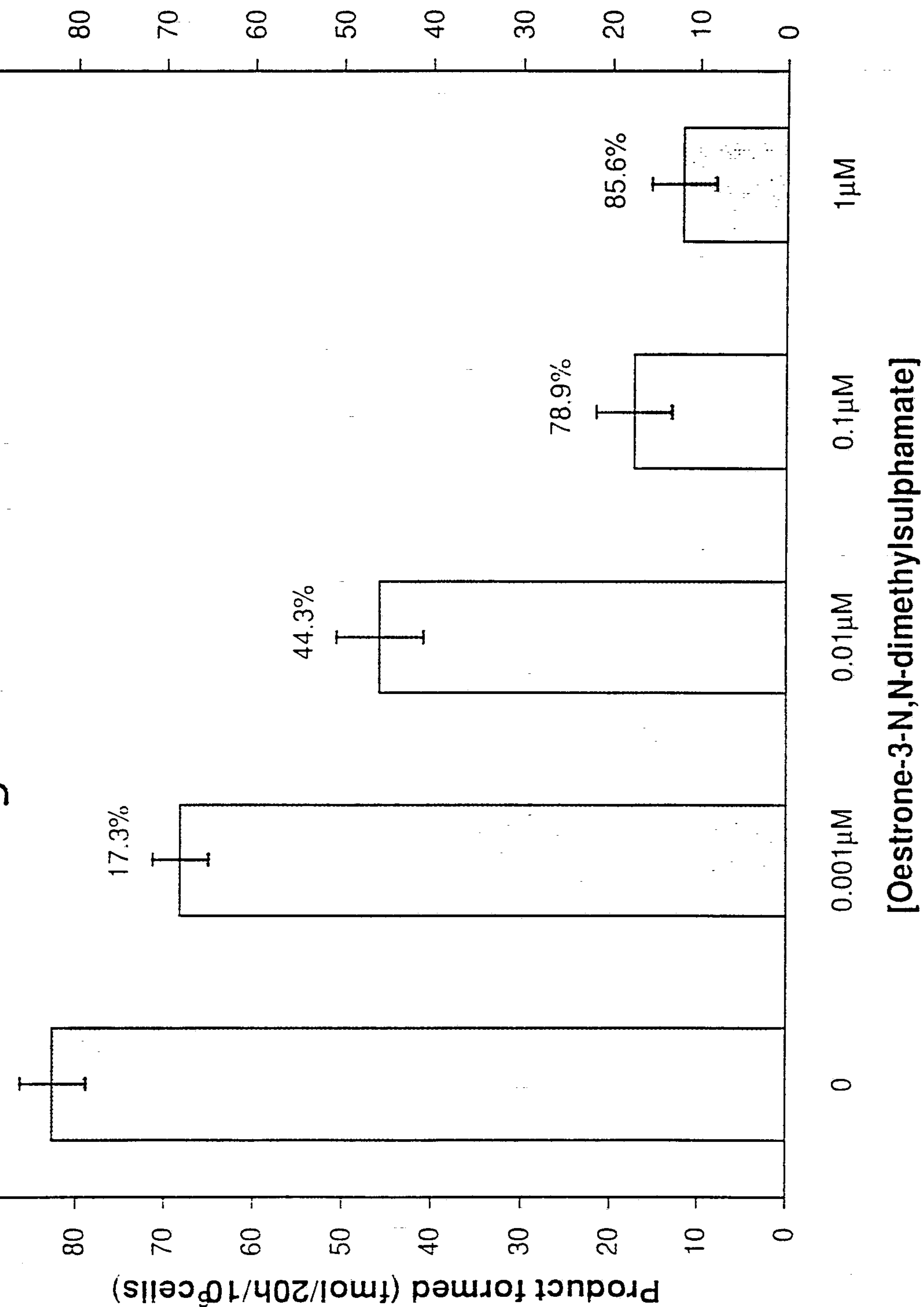

Product formed (fmol/20h/10^6cells)
0
10
20
30
40
50
60
70
80
90
17.3%
44.3%
78.9%
85.6%
0
0.001µM
0.01µM
0.1µM
1µM
[Oestrone-3-N,N-dimethylsulphamate]

Fig. 4

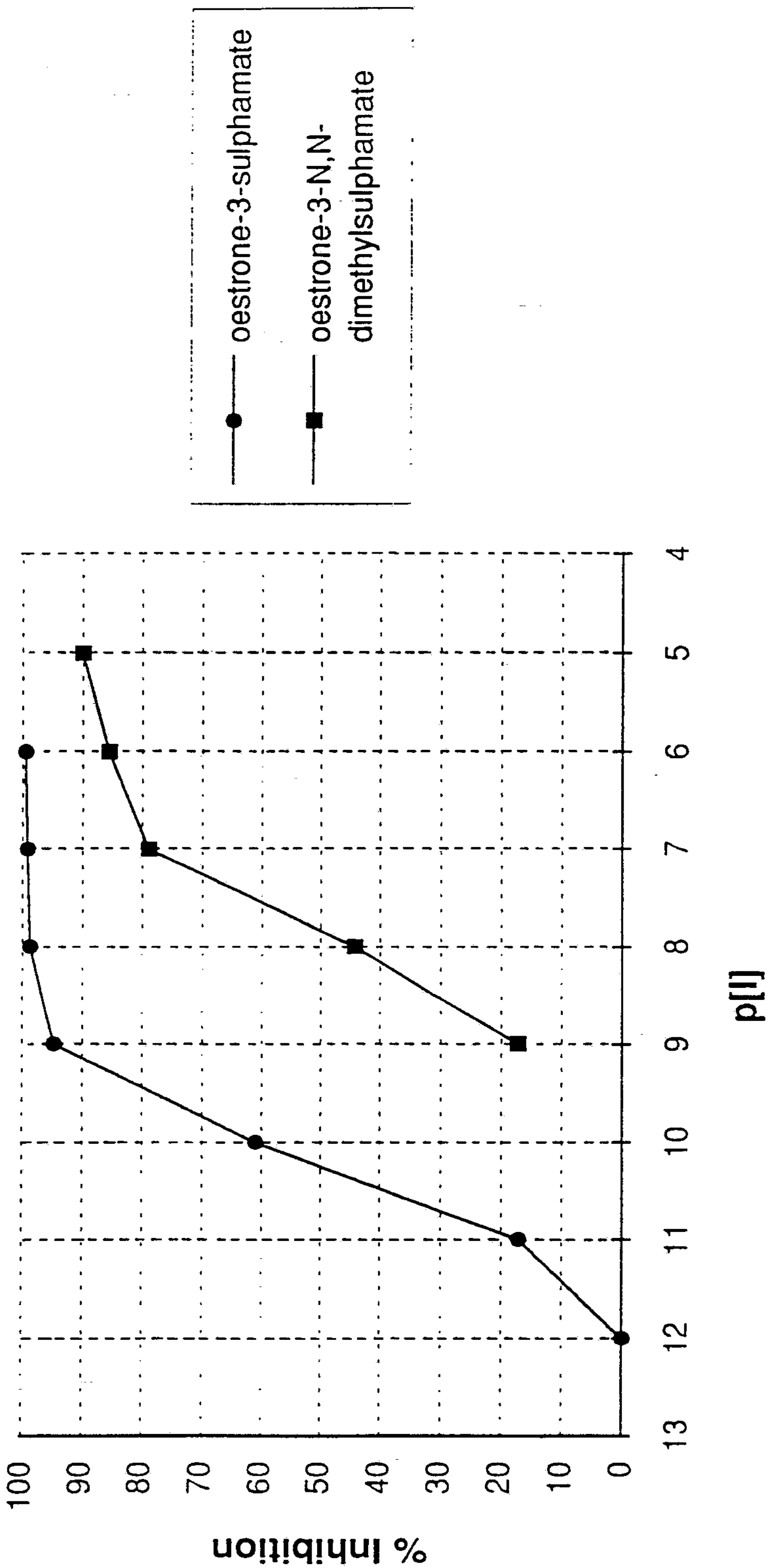
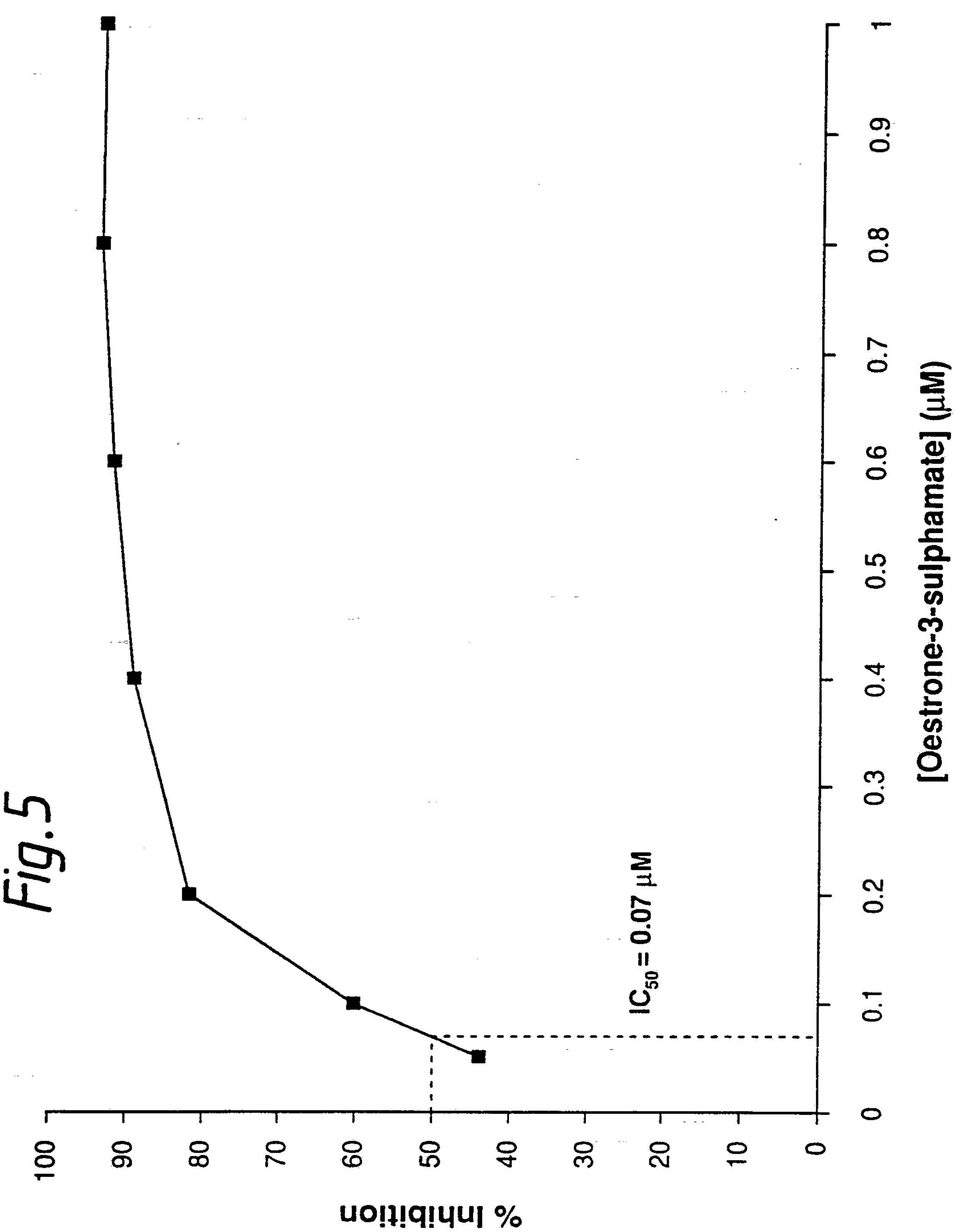

oestrone-3-sulphamate
oestrone-3-N,N-dimethylsulphamate
% Inhibition
100
90
80
70
60
50
40
30
20
10
0
p[I]
13
12
11
10
9
8
7
6
5
4

Fig. 5


% Inhibition
100
90
80
70
60
50
40
30
20
10
0
IC_{50} = 0.07 μM
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
[Oestrone-3-sulphamate] (μM)

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/01587

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07J41/00; A61K31/565

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07J ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category°	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X A	PHARMAZIE vol. 30, no. 1, January 1975, BERLIN DD pages 17 - 21 S. SCHWARZ ET AL 'Seroide. 15 Mitteilung : Sulfonyloxyderivate von Oestrogenen' see the whole document, particularly page 18, column 1, lines 20-37 ---	1-7 11
X A	DD,A,114 806 (S. SCHWARZ) 20 August 1975 see the whole document, particularly example 10 ---	1-7 11
X	GB,A,1 398 026 (VEB JENAPHARM) 18 June 1975 see the whole document, particularly examples 2-6 and 18 --- -/--	1-7

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

1

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
02 NOVEMBER 1992	23.11.92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P. WATCHORN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	ZEITSCHRIFT FÜR CHEMIE vol. 14, no. 1, 1974, LEIPZIG, DE pages 15 - 16 S. SCHWARZ ET AL 'Steroidsulfamate'	1-7
A	see the whole document ---	11
A	RESEARCH ON STEROIDS vol. 5, 1973, ROME, IT pages 73 - 78 J. TOWNSLEY 'Structure Activity Correlations for Steroid Inhibition of Human Placental Steroid 3-Sulfatase' see the whole document -----	1,11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB92/01587

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 12-16 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compounds.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The subject matter of claims 1,2 and 12 (and thus claims 6-8 and 11 in so far as they depend on calim 1 and 2) defines structural features of the compounds of these claims in functional terms resulting in a lack of clarity(Art.6 PCT)

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9201587 SA 63946

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 02/11/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DD-A-114806		None	
GB-A-1398026	18-06-75	None	

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82